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ORIGINAL RESEARCH ARTICLE

The comparative virulence of an atoxigenic strain of *Aspergillus flavus* (Eurotiales: Trichocomaceae) and the commercial ICIPE 69 *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) to the bean leaf beetle *Ootheca mutabilis* (Coleoptera: Chrysomelidae)

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Abstract
The recent isolation of an atoxigenic strain of *Aspergillus flavus* (Link) (Eurotiales: Trichocomaceae), which was virulent to all stages of *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae) has generated interest to investigate virulence of the entomopathogenic fungus against other crop pests, and how it compares with established commercial biopesticides. In this study, we tested the pathogenicity and virulence of the atoxigenic *A. flavus* strain to *Ootheca mutabilis* (Sahlberg) (Coleoptera: Chrysomelidae) in the laboratory compared to the commercial *Metarhizium anisopliae* (Metschnikoff Sorokin) (Hypocreales: Clavicipitaceae; OD®, ICIPE 69). Serial dilutions of both entomopathogenic fungi (1×10^6–1×10^9 conidia/ml) were evaluated against adult *O. mutabilis*. Distilled water and cypermethrin 5% EC were included as controls. The treatments were replicated four times and the bioassay was repeated once. Both fungi at 1×10^9 conidia/ml killed 100% of tested *O. mutabilis* within seven days in the 1st and 2nd trials. The concentration of both fungi that killed 50% of *O. mutabilis* was the same in both trials (5.3×10^6 conidia/ml). The time taken by *A. flavus* and ICIPE 69 to kill 50% of *O. mutabilis* was not significantly different in the 1st trial (3.4 ± 0.1 days and 2.9 ± 0.4 days, respectively). However, ICIPE 69 took a significantly shorter time (2.1 ± 0.4 days) than *A. flavus* (3.6 ± 0.2 days) to kill 50% of *O. mutabilis* in the 2nd trial. We concluded that *A. flavus* is comparably as pathogenic and virulent (in terms of cumulative mortality and LC_{50}) against *O. mutabilis* as ICIPE 69, and therefore both fungi may be viable biopesticides for commercialization against this pest.

Keywords Biopesticides · Common bean · Entomopathogenic fungi · *Phaseolus vulgaris*

Introduction

Bean leaf beetles, *Ootheca* spp. (Coleoptera: Chrysomelidae) are important indigenous agricultural field pests of the common bean *Phaseolus vulgaris* (Linnaeus) (Fabales: Fabaceae) in Sub-Saharan Africa (SSA) (Paul et al. 2007; Bazie et al. 2019). Two species in the genus *Ootheca*, namely *O. bennigseni* (Weise) and *O. mutabilis* (Sahlberg) are common in eastern and southern Africa (Abate and Ampofo 1996; Kankwatsa 2018). These beetles attack the roots, leaves, flowers and bean pods, causing variable damage depending on the location; season and environmental conditions (Grobbelaar 2008; Mwanauta et al. 2015). Attack on roots by the early instar larvae may go unnoticed but the older larvae feed on lateral roots, causing wilting and premature senescence in bean plants. Emergence of adults usually coincides with germination of host plants at the onset of the rainy season. In case of a prolonged dry season, teneral adults undergo diapause until the onset of the next rainy season when they emerge and start feeding on leaves of the newly planted legumes. Adult damage to the growing shoot of the young seedling destroys the entire plant. These beetles continue to feed on the foliage between veins

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of growing legume plants, and cause extensive defoliation. Grain yield losses of the common bean attributed to *Ootheca* spp. range from 18% to 31% and complete crop loss can occur under severe infestation (Karel and Rweyemamu, 1984; Mwanauta et al. 2015; Obanyi et al. 2017).

Common bean farmers rely on cultural practices, botanicals and synthetic pesticides to control *Ootheca* spp. (Paul et al. 2007; Buruchara et al. 2010; Bazie et al. 2019). The cultural methods for controlling these pests include post-harvest tillage, crop rotation with non-host plants e.g. maize or sunflower, genetic mixtures and delayed sowing of common bean. These methods are cheap, dependable and specific, and do not possess detrimental side effects of synthetic pesticides. However, cultural methods do not provide complete economic control of the pests; their efficacy is difficult to assess; they require careful timing and long-term planning, and some are labor intensive (e.g. post-harvest tillage) to small-scale resource poor farmers who rely on the hand-hoe for land preparation. The botanical pesticides that deter *Ootheca* spp. and reduce damage to common beans include an aqueous extract from vernonia *Vernonia lasiopus* var. *iodocalyx* (Hoffmann) (Asterales: Asteraceae) leaves and Neem *Azadirachta indica* (De Jussieu) (Sapindales: Meliaceae) seed extracts. These botanical pesticides have low preparation and use costs, selective in action, and minimally toxic to humans, livestock and beneficial non-target organisms (Umoetok et al. 2009). However, botanical pesticides have short residual periods, slow effect on the pests, require frequent spraying and availability of their raw materials is seasonal. The active ingredients in botanical pesticides are liable to decomposition, their preparations have complex compositions and are not easy to standardize (El-Wakeil 2013).

Foliar application of synthetic pyrethroids for example, cypermethrin EC and lambda cyhalothrin EC are effective in controlling *Ootheca* spp. (Schwartz and Corrales, 1989; Paul et al. 2007; Tembo et al. 2018). Nevertheless, regular application of broad-spectrum pesticides are not recommended since their repeated use may result into resistance across a wider spectrum of common bean pests (Srinivasan 2014). Additionally, chemical pesticides can cause contamination of ground water and riparian habitats, outbreaks of secondary pests, decrease in biodiversity, and safety risks for humans, and wild and domestic animals (Dolinski and Lacey 2007; Mwanauta et al. 2015). Therefore, crop protection focus has shifted to integrated pest management (IPM), with emphasis on the use of biological control agents such as entomopathogens (Yubak Dhoj et al. 2008; Tesfaye and Seyoum 2010).

Several commercial products based on different species in genera of entomopathogenic fungi (EPF) such as, *Metarhizium, Beauveria, Verticillium, Nomuraea, Aspergillus, Entomophthora, Paecilomyces* and *Neozygites* have been formulated; targeting important pests and vectors (Sileshi et al. 2013; Bhan et al. 2015; Erler and Ates 2015; Bohara et al. 2018). Entomopathogenic fungi are eco-friendly alternatives for managing insect pests of public health and agricultural importance (Cravanzola et al., 1997; Charnley and Collins, 2007; Teshome and Tefera 2009; Farooq and Freed 2016). In the genus *Metarhizium*, *M. anisopliae* based biopesticides are frequently used to manage many insect pests including: *Maladera affinis* (Blanchard) (Coleoptera: Scarabaeidae); *Alaus oculatus* (Linnaeus) (Coleoptera: Elateridae) (Krishnamurthy et al. 1989); *Holotrichia serrata* (Fabricius) (Coleoptera: Scarabaeidae) (Cassell and Kuhar 2009) and some orthopterans (locusts and grasshoppers) (Magalhaes et al. 2000). In the genus *Aspergillus, A. flavus* has S- and L-strains or morphotypes, depending on sclerotia morphology (Dorner 2004; Donner et al. 2010; Ohkura et al. 2018). The L-strain produces fewer large sclerotia and abundant conidia, with varying aflatoxin-producing capabilities and most of them are atoxigenic (i.e., do not produce aflatoxins). The S-strain of *A. flavus* produces larger quantities of small sclerotia and more aflatoxins than the L-strain, restraining the former from application in vector and insect pest control (Powell et al. 2013). Most L-strain isolates of *A. flavus* are basic active agents in biocontrol products that are used to manage aflatoxin contamination (Probst et al. 2010). The atoxigenic L-strain of *A. flavus* is used as a biopesticide against *Anopheles stephensi* (Liston) (Diptera: Culicidae) and *Culex quinquefasciatus* (Say) (Diptera: Culicidae) (Bhan et al. 2015). *Aspergillus flavus* was reported effective against the red spider mite *Oligonychus coffeae* (Nietner) (Acari: Tetranychidae), a serious pest of tea in India (Mazid et al. 2015). Mukasa et al. (2018) isolated an atoxigenic strain of *A. flavus* in Uganda which was highly lethal to all development stages of *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae: Scolytinae), causing mortalities of between 70% and 100% in the laboratory and 71% and 79% in the field; and suggested testing the entomopathogen against other species of insect pests. This isolate was, therefore, worthy of evaluation for control of important pests such as *O. mutabilis,* in comparison to known commercial biological control agents.

In this study, we investigated the comparative pathogenicity and virulence of the atoxigenic *A. flavus* and the commercial entomopathogenic biopesticide ICIPE 69, *M. anisopliae,* against *O. mutabilis* in the laboratory with a view of evaluating the potential of commercializing the isolate of *A. flavus* against this pest. We chose *O. mutabilis* because our recent studies (unpublished) showed this species of bean leaf beetles to be more widely spread and damaging to the common bean in Uganda than its sister species *O. bennigseni*.

Materials and methods

Insects

The 1st batch of *O. mutabilis* was collected during March to May 2017 planting season from Manibe sub-county in Arua

district (03°05′20″N, 30°57′24″E and 1181 m above sea level (masl)); whereas the second batch was collected during March to May 2018 planting season from Agweng sub-county in Lira district (02°29′08″N, 32°54′35″E and 1084 masl). Both sub-counties were selected in consultation with agricultural extension officers and local farmers, based on heavy infestation of common bean gardens by the beetles. In the two sub-counties, *O. mutabilis* were collected from common bean gardens where pesticides had not been used during the season. Adult beetles were collected by handpicking and placed in translucent plastic containers (150 mm height × 60 mm top internal diameter × 40 mm bottom internal diameter; Crown Industries Ltd., Nairobi, Kenya). The containers were ventilated by creating three circular windows measuring 30 mm in diameter on opposite sides and on the lid of the container. The windows were fitted with muslin cloth to prevent beetles from escaping. Fresh tender common bean leaves from the gardens where beetles were collected were added to the containers as food. The top windows of the containers were covered with moistened wads of cotton (Carex, Inik Industries Ltd., Wakiso, Uganda) to maintain RH at between 40% and 60% (Beck and Blumer 2011). The translucent plastic containers (with beetles inside) were transported to the Entomology and Plant Pathology Laboratory at the National Crops Resources Research Institute (NaCRRI), Namulonge, Wakiso, Uganda (00° 31′ 30″N, 32° 36′ 54″E and 1127 masl) from where they were sorted and identified according to Kortenhaus and Wagner (2010). Sorted beetles were placed into clean translucent plastic containers described above and provided with fresh tender common bean leaves (NABE 16, raised in the legume sunscreen at NaCRRI). All adult insects were eligible for the bioassay, regardless of sex.

Entomopathogenic fungi

A commercial oil-formulated biopesticide *M. anisopliae,* (Real *M. anisopliae* 69 OD® - ICIPE 69, Real IPM Company, Kenya Limited) manufactured to control whiteflies, mealybugs, thrips and snout beetles; and a soil-borne isolate of atoxigenic *A. flavus* (L- strain) from Mubende district in Uganda (GPS coordinates kept private), reported to be highly lethal to all growth stages of *X. compactus* (Mukasa et al. 2018) were used in this study. The two entomopathogenic fungi were passaged through adult *O. mutabilis* to prevent attenuation (Butt and Goettel 2000; Tumuhaise et al. 2015) and loss of virulence, which would result from maintaining the fungi on artificial substrate (Safavi 2012). The spores from mycosed cadavers of *O. mutabilis* were propagated on Potato Dextrose Agar (PDA) (Oxoid, CMO 139, England) medium and incubated at room temperature for 20 days to obtain fungal cultures for subsequent bioassays.

Preparation of conidial suspensions and assessment of their viability

For both *A. flavus* and ICIPE 69, conidia from a 20-day old culture (described above) were harvested with a sterile loop needle and suspended in an aqueous solution of 0.05% Tween 80 (Himedia Laboratories Pvt. Ltd., 23 Vadhan Industries, Mumbai, India) as a wetting agent and sterile distilled water in 10 ml glass bottles with 10 glass beads (3 mm). The fungal suspensions in glass bottles were vortexed on a mechanical shaker (5 min at 700 oscillations/min) to break-up the conidial clumps, and filtered through a double layer of sterile muslin cloth to remove coagulated media (Slade et al. 1987; Sileshi et al. 2013).

The concentration of conidia in the filtrate was estimated using a heamocytometer (CAT. No. 1103, China) under a light microscope (Axioplan, Carl Zeiss Microscopy, D07740-Jena, Germany) (Goettel and Inglis 1997; Inglis et al. 2012). Briefly, the conidial concentration was quantified by loading a uniform suspension of conidia into the coverslip-affixed chambers of the haemocytometer. The haemocytometer was placed under the microscope at ×10 objective to facilitate localization of the grid, and counting started two minutes after the conidia had settled. The focus was on four large squares (consisting of 16 small squares) at each corner. Conidia in each of the four squares and at the right hand and bottom boundary lines were counted while those on the left border and upper boundary lines were skipped as per the classical procedure (Araujo et al. 2004; Pasco et al. 2016; Rishi et al. 2016). The average number of conidia for the four squares was calculated and used to estimate the conidia/ml of suspension (average conidia count per large square × 10^4).

Conidial viabilities of *A. flavus* and ICIPE 69 were tested according to Mohammed (2013). A standard conidial suspension of 3×10^6 conidia/ml of each entomopathogenic fungus was prepared. For each conidial suspension, an aliquot of 0.1 ml of the 3×10^6 conidia/ml suspension was placed on PDA plate with a sterile glass rod; sealed with parafilm (Penchiney, Menasha W154952, India) and incubated in complete darkness at 27 °C and 75% RH for 24 h (Teshome and Tefera 2009; Huang et al. 2012; Mazid et al. 2015). Germination was terminated by adding a drop of 0.5% formaldehyde (Loba Chemie Pvt. Ltd., Mumbai, India) onto the plate. Two sterile microscope cover slips (Chance Propper Ltd., West Mids, England) were placed on the plate, and germination percentage was determined by counting 100 spores under the cover slip on the plate under a light microscope at ×400 magnifications. Only conidia with a germ tube at least twice the size of the spore were considered to have germinated (Inglis et al. 2012). The procedure was replicated four times for each entomopathogenic fungus and the mean spore germinations were computed as 92.4 ± 3% and 94.6 ± 2% for *A. flavus* and ICIPE 69, respectively. These levels of viability

were all way higher than 80%, which is sufficient to cause substantial mortality on target pests (Ekesi et al. 1998; Teshome and Tefera 2009).

Preparation of conidial dilutions and experimental controls

The surfaces of 20-day old cultures of *A. flavus* and ICIPE 69 were harvested using a sterile loop needle and separately suspended in 10 ml glass bottles containing 10 glass beads and aqueous solution of 0.05% Tween 80. The suspensions were vortexed and filtered through double layers of sterile muslin cloth. A stock suspension of 1×10^9 conidia/ml was estimated for each entomopathogenic fungus using a haemocytometer. Serial dilutions of 1×10^8, 1×10^7, and 1×10^6 conidia/ml of distilled water were made from the stock suspensions of each test entomopathogenic fungus. A standard chemical pesticide, CyperLacer® (cypermethrin 5% EC; Agrochemicals Pvt., Ltd. Gujarat, India) and sterile distilled water (containing 0.05% Tween 80 only) were prepared as controls. Cypermethrin 5% EC was selected because it's commonly used for controlling field insect pests of the common bean in Uganda. The pesticide - cypermethrin 5% EC- was prepared by mixing 75 ml in five liters of water as per the manufacturer's recommendation.

Dose-response bioassay

The 10 treatments (four from each entomopathogenic fungus, one for distilled water and one for the chemical pesticide) were replicated four times. Each treatment was applied on 10 adult *O. mutabilis* (irrespective of sex) in a translucent circular plastic container (Everfresh No. 4 (530), Kenpoly, Kenya) measuring 110 mm diameter × 65 mm height. The containers were lined at the bottom with a layer of moist cotton wool beneath a circular filter paper (Whatman No. 1, 1001–090) to absorb excess suspension, and covered with muslin cloth on top. Aliquots (2.5 ml) of each conidial suspension were directly sprayed on the beetles in the containers, wetting them completely, using handheld plastic trigger sprayers (Yuyao Lucky Commodity Co., Ltd., China) of 1.5 l capacity (Butt and Goettel 2000; Sanjaya et al. 2013; Mukasa et al. 2018). Similarly, beetles in each of the control containers were separately sprayed with 2.5 ml of (a) sterile distilled water and (b) CyperLacer®. Separate sprayers were used for each treatment to prevent cross-contamination. For each fungal suspension, lower concentrations were consecutively sprayed first to minimize leftover effects on concentrations. The beetles were provided with tender common bean leaves from the sunscreen at NaCRRI daily throughout the experiments. The experiments were conducted at 25 ± 4 °C and 60 ± 5% RH in the Entomology and Plant Pathology Laboratory at NaCRRI. The number of dead beetles in each treatment was recorded daily for seven days, starting 24 h after spraying (Burges, 1981; Tumuhaise et al. 2015; Rishi et al. 2016).

To confirm fungal infection in *O. mutabilis,* cadavers from *A. flavus* and ICIPE 69 treated insects were separately surface sterilized with 0.2% sodium hypochlorite solution (Orbit Chemical Industries Ltd. Nairobi, Kenya) for three seconds; washed twice in sterile distilled water and placed individually on moist filter paper in fresh sterile Petri dishes (100 mm × 17 mm). They were incubated at 27 °C for seven days. After three to five days, mycelia were observed growing out of the cadavers. The retrieved mycelia were cultured on PDA medium for 20 days, and compared with those obtained from passaging of the two EPF through the beetles (i.e. colony growth and appearance, pigmentation at the reverse of PDA plate and diffusion of pigmented metabolites into the Potato Dextrose Broth medium). The bioassay was repeated once.

Statistical analysis

Since mortality in the chemical pesticide treatment was 100% within the first 24 h, and no mortality was recorded in the control treated with sterile distilled water in the 1st and 2nd trials; data from the two controls were not included in the statistical analyses (Tesfaye and Seyoum 2010; Pasco et al. 2016). For each trial, cumulative mortalities of *O. mutabilis* from replicates of *A. flavus* and ICIPE 69 at the highest concentration (1×10^9 conidia/ml) by day seven were both 100%; therefore, these data did not require statistical comparison. To generate the LC_{50} (conidial concentrations that were lethal to 50% of *O. mutabilis*) on the last day and LT_{50} (lethal time at which 50% of *O. mutabilis* were killed) at the highest conidial concentration for each entomopathogenic fungus, data on dead (0) and alive (1) adults of *O. mutabilis* for each EPF in the two trials were subjected to Generalized Linear Model (GLM) procedure with binomial distribution error and logit link (Warton and Hui 2011). The ratios of residual deviances to degrees of freedom from the GLM outputs approximated to 1 in all cases, indicating appropriateness of the models. The function 'dose.p' from the 'MASS' library was used to produce LC_{50} and LT_{50} estimates, which were compared between the two EPF by a Two Sample t-test at $\alpha = 0.05$. All the analyses were carried out in R-statistical computer software version 3.4.3 (R Development Core Team 2017).

Results

Mortality of *Ootheca mutabilis* in the control treatments

The mortalities of *O. mutabilis* in the control treatments of the standard chemical pesticide (CyperLacer®) were 100% in all replicates in both trials after 24 h. However, no mortalities

were recorded in control treatments of sterile distilled water (containing 0.05% Tween 80) in all treatments in both trials after seven days.

Pathogenicity of *Aspergillus flavus* and ICIPE 69 against *Ootheca mutabilis*

The cumulative mortalities of *O. mutabilis* treated with *A. flavus* and ICIPE 69 at 1×10^9 conidia/ml on the 7th day in the 1st and 2nd trials were 100%.

Median lethal concentrations of *Aspergillus flavus* and ICIPE 69 against *Ootheca mutabilis*

The median lethal concentrations of *A. flavus* and ICIPE 69 on the 7th day (approximately 5.3×10^6 conidia/ml for both entomopathogenic fungi) were not significantly different in both trials ($t=0.596$, $df=6$, $P=0.573$ and $t=0.289$, $df=6$, $P=0.782$ for trials 1 and 2, respectively) (Fig. 1).

Median lethal time of *Aspergillus flavus* and ICIPE 69 against *Ootheca mutabilis*

Whereas the LT_{50} for *A. flavus* and ICIPE 69 against *O. mutabilis* at the highest entomopathogenic fungal conidial concentrations were not significantly different in the 1st trial ($t=1.103$, $df=6$, $P=0.313$), the LT_{50} of *A. flavus* (3.6 ± 0.2 days) was significantly longer than that of ICIPE 69 (2.1 ± 0.4 days) in the 2nd trial ($t=3.331$, $df=6$, $P=0.016$) (Fig. 2).

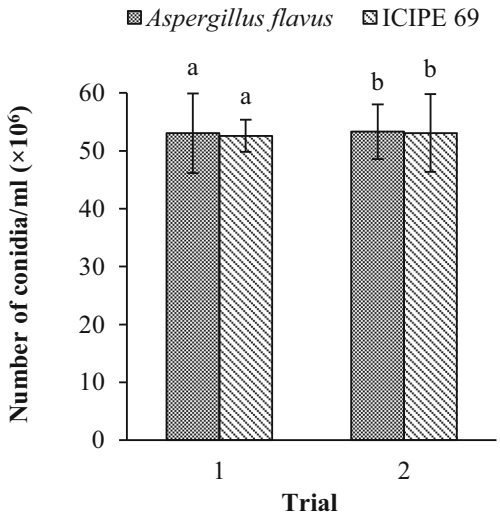

Fig. 1 Mean ± standard error of the mean (SEM) of LC_{50} estimates for *Aspergillus flavus* and ICIPE 69 against *Ootheca mutabilis*. Same letters on bars within a trial indicate no significant differences between means (Two Sample t-test; $\alpha=0.05$)

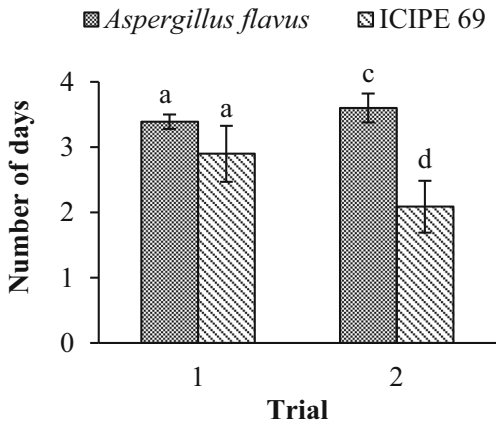

Fig. 2 Mean ± SEM of LT_{50} for *Aspergillus flavus* and ICIPE 69 against *Ootheca mutabilis*. Different letters on bars within a trial indicate significant differences between means (Two Sample t-test; $\alpha=0.05$)

Discussion

The standard pesticide (cypermethrin EC 5%) caused maximum mortalities (100%) of *O. mutabilis* in both trials after 24 h of application. This is an indication of the quick knock down and kill properties of broad-spectrum synthetic insecticides like cypermethrin. This finding concurs with that of Reynolds et al. (2017) in which topical application of cypermethrin EC on the Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) caused maximum mortality (100%) in the first 24 h. Cypermethrin EC is used in the management of *Ootheca* spp. in leguminous crops in Tanzania (Tembo et al. 2018). As is common with other insect pest species, cypermethrin EC is an important option in reducing larval populations of the fruit borer *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) and the pea pod borer *Etiella zinckenella* (Treitschke) (Lepidoptera: Pyraloidea) in cowpeas in Egypt (Mahmoud 2011). In India, aphids *Myzus persicae (*Sulzer) (Hemiptera: Aphididae), whiteflies *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and thrips *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae) that attack tomatoes *Lycopersicon esculentum* Miller (Solanales: Solanaceae) are managed by applying cypermethrin EC (Wagh et al. 2017). In California, United States of America (USA), a number of agricultural crop pests including the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae); pea leafminer *Liriomyza langei* (Frick) (Diptera: Agromyzidae); American serpentine leafminer *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) and vegetable leafminer *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae) are managed with cypermethrin EC (Shimat et al. 2017). In Cameroon, cowpea pests *O. mutabilis*; flower thrips, *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae); Aphids, *Aphis craccivora* (Koch) (Hemiptera: Aphididae); legume pod borer, *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae) and

pod sucking bugs - *Clavigralla tomentosicolis* (Stal) (Hemiptera: Coreidae) and *Anoplocnemis curvipes* (Fabricius) (Hemiptera: Coreidae)- are managed with cypermethrin EC (Djidjonri et al. 2019).

However, Thatheyus and Selvam (2013) and Srinivasan (2014) caution against reliance on synthetic pyrethroids in the control of insect pests because they are non-selective and, therefore, their repeated use may cause resistance across a range of common bean pests. Cypermethrin EC is detrimental to the ladybird beetle *Coccinella magnifica* (Latreille) (Coleoptera: Coccinellidae), which is a natural enemy of bean aphids *Aphis* spp. (Hemiptera: Aphididae) and bean flies *Ophiomyia* spp. (Diptera: Agromyzidae). Cypermethrin is equally harmful to predatory spiders *Deinopis* spp. (Araneae: Deinopidae) of *Ophiomyia* spp. and parasitoid flies Istocheta spp. (Diptera: Tachinidae) of coleopterans, including *Ootheca* spp. (Mohan et al. 2013; Wagh et al. 2017). This highlights the need to develop an IPM strategy for *O. mutabilis* control without or with limited use of synthetic pesticides.

There were no mortalities of *O. mutabilis* in the second control of sterile distilled water containing 0.05% tween 80 during the first seven days of the experiment in both trials. Absence of mortality in the water control has been reported in several researches, for example, no mortalities were reported in the water control within the first 10 days of treating the maize weevil *Sitophilus zeamais* (Mostch) (Coleoptera: Curculionidae) with *B. bassiana* and *M. anisopliae* (Teshome and Tefera 2009). Additionally, Pasco et al. (2016) reported no mortalities in the water control in 14 days during the assessment of the efficacy of topical, leaf residue, and soil drench applications with *Isaria fumosorosea* Wise (Hypocreales: Cordycipitaceae) blastospores (Ifr strain 3581) for management of citrus root weevils *Diaprepes abbreviatus* (Linnaeus) (Coleoptera: Curculionidae). Absence of mortalities in the control without a killing agent indicates that the beetles are hardy and, therefore, may perpetuate in the field unless controlled. This finding may explain the high abundance of *Ootheca* spp. during rainy periods, in coincidence with germinating legumes (Buruchara et al. 2010). The teneral adults of *O. mutabilis* can also withstand harsh field conditions of prolonged droughts by undergoing an obligatory diapause until onset of the next rainy season when they emerge and start feeding on leaves of newly planted legumes. However, cases of mortalities in experiments involving water controls are also commonly reported in literature, for example: Sileshi et al. (2013) recorded 6.67% mortality in termites *Macrotermes* (Isoptera: Termitidae) during an evaluation of *M. anisopliae* and *B. bassiana* against these pest in six days; Kirubakaran et al. (2015) recorded about 5% mortality in *C. quinquefasciatus* in seven days of the experiment; and Bayissa et al. (2017) recorded between 15% and 20% mortality in the water control treatments in the bioassay involving the three aphids *Aphis gossypii* (Glover), *Brevicoryne brassicae* (Linnaeus) and *Lipaphis pseudobrassicae* (Davis) (Hemiptera: Aphididae).

In both trials, the cumulative mortalities of *O. mutabilis* treated with 1×10^9 conidia/ml of both *A. flavus* and ICIPE 69 on the 7th day were 100%. This implies that both EPF were equally pathogenic and can hence be potential biopesticides in managing *O. mutabilis*. This is consistent with Fernandes et al. (2012) that arachnids and insect pests including coleopterans are susceptible to fungal infections, for example, *M. anisopliae* infects a wide range of beetles, including the white grub of *Hoplia philanthus* (Füessly) (Coleoptera: Scarabaeidae) (Ansari et al. 2006) and the rhinoceros beetle *Oryctes rhinoceros* (Linnaeus) (Coleoptera: Scarabaeidae) (Bedford 2014). Other agricultural pests that are susceptible to *M. anisopliae* infections include *B. brassicae* and *L. pseudobrassicae* (Bayissa et al. 2017); *M. vitrata* (Tumuhaise et al. 2015) and polyphagous thrips (Wu et al. 2018). The atoxigenic strains of *A. flavus* are commercially registered products in USA for managing aflatoxins in pre- and post-harvested cotton, peanut and corn through competing and displacing toxigenic strains (Dorner 2004; Abbas et al. 2011). However, the same strains are reportedly pathogenic to coleopterans, such as the shot-hole borer *Scolytus nitidus* (Schedl) (Coleoptera: Curculionidae: Scolytinae) (Buhroo et al. 2002); pupae of the small hive beetle *Aethina tumida* (Murray) (Coleoptera: Nitidulidae) (Rong et al. 2004); the almond bark beetles *Scolytus amygdali* (Guerin-Meneville) (Coleoptera: Curculionidae: Scolytinae) (Asma et al. 2017) and the coffee twig borer *X. compactus* (Mukasa et al. 2018). Larvae of the southern house mosquito (*C. quinquefasciatus*) are also susceptible to *A. flavus* (Bhan et al. 2015). The current study broadens the spectrum of insect pests that are susceptible to *A. flavus*. It also provides potential for commercial production of atoxigenic *A. flavus* for *O. mutabilis* control. However, further research is required to determine the pathogenicity of the entomopathogenic fungus to all developmental stages of *O. mutabilis* as well as its efficacy against the pest in the field.

Based on dose-mortality relationships, the LC_{50} estimates for *A. flavus* and ICIPE 69 were comparable indicating that both EPF are equally virulent to *O. mutabilis*. Besides probable innate similarities in the virulence of the two EPF, similar laboratory conditions (such as relative humidity, temperature and light) under which they were tested may also explain this finding. These conditions are vital determinants of the rates and quantities of fungi that germinate, sporulate and infect the pests (Goettel and Inglis 1997; Sanjaya et al. 2013). The LC_{50} estimates depend on the number of infectious propagules in contact with the host cuticle. Propagules have to reach susceptible sites on the host cuticle, for example under the elytra and the intersegmental membranes (Bedford 2014). The viability of infectious propagules on the host cuticle is interrupted by

preening, ecdysis and basking in the sun. Insect vigor, handling and rearing conditions affect the susceptibility of the insect to viable propagules (Butt and Goettel 2000; Ibrahim et al. 2015). To our knowledge, this is the first report of the virulence of *A. flavus* and ICIPE 69 against *O. mutabilis*; which broadens the spectrum of control options against this important common bean pest. However, as noted above, further research is required to determine the virulence of these entomopathogens on other developmental stages of *O. mutabilis,* and under field conditions prior to their endorsement for use in the management of the pest.

The LT_{50} of ICIPE 69 was significantly shorter than that of *A. flavus* in the 2nd trial. Intra- and inter-specific differences in virulence is common among EPF (Ekesi et al. 1998; Wu et al. 2018). The high virulence of ICIPE 69 among other isolates of *M. anisopliae* and those of *Beauveria bassiana* was reported for *M. vitrata* (Tumuhaise et al. 2015). It is probable that ICIPE 69 produces superior toxins that are responsible for faster death of *O. mutabilis* than those produced by *A. flavus*. Nonetheless, the delay to kill *O. mutabilis* by *A. flavus* may be of less significance if the incapacitating effect of infection can reduce the beetle's capacity to damage the common bean. The ability of pests to damage crops reduces drastically after treatment with infectious propagules of EPF (Inglis et al. 2012; Safavi 2012; Bedford 2014). The various researchers attribute anti-feedant effects of EPF to the production of toxins and mechanical disruption of insect tissues by hyphal growth.

In conclusion, the current study demonstrates that *A. flavus* and ICIPE 69 are equally pathogenic and virulent (in terms of cumulative mortalities and concentrations required to kill 50% of the pests) to *O. mutabilis*. However, the two EPF require different times to kill 50% of this pest. Both EPF are potential alternatives to the use of synthetic pesticides in the management of *O. mutabilis*. This provides a leeway to develop atoxigenic *A. flavus* into a commercial biopesticide for *O. mutabilis* control. We recommend further studies on the pathogenicity and virulence of *A. flavus* and ICIPE 69 involving other developmental stages of *O. mutabilis,* and under field conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This study did not involve use of human and/or vertebrate animals.

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